IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: **YU et al.**

Application Serial No.: 09/333,966 Art Unit: 1646

Filed: June 16, 1999 Examiner: Ulm, J.

For: Death Domain Containing Receptors Attorney Docket No.: **PF267D1**

DECLARATION OF THI-SAU MIGONE UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Thi-Sau Migone Ph.D., hereby declare and state as follows:

1. I am currently employed as a Senior Scientist at Human Genome Sciences, Inc. (HGS), which I understand to be the assignee of the above-captioned patent application (the '966 Application). In 1991, I received my Ph.D. from the University of Pavia, Italy in Biochemistry. From 1991 to 1992, I served a professional apprenticeship at the Ospedale San Raffaele in Milan, Italy, where I carried out research in the Laboratory of AIDS-Immunopathogenesis. From 1992 to 1993, I was a guest researcher at the National Institute of Allergy and Infectious Disease in Bethesda, Maryland, where I carried out research in the Laboratory of Molecular Microbiology. From 1993 to 1996, I was a Special Volunteer-Fogarty Visiting Fellow at the National Heart, Lung, Blood Institute in Bethesda, Maryland, where I carried out research in the Laboratory of Molecular Immunology. From 1997 to 1999, I was a Postdoctoral Fellow at the DNAX Research Institute in Palo Alto, California, where I carried out research in the Department

of Cell Signaling. Since 1999 I have been employed by HGS, where my research has included both directly carrying out and supervising the discovery, recombinant expression, isolation, and biochemical and biological characterization of human therapeutic proteins. I have co-authored over twenty articles that have been published in peer-reviewed scientific journals. A copy of my curriculum vitae is attached hereto as Exhibit A.

2. I have been shown and have examined U.S. Patent Application No. 09/333,966 (the '966 Application), captioned above, which I understand was filed on June 16, 1999. I will refer to the '966 Application as "the Application."

3. I am the first named author of the publication entitled "TL1A Is a TNF-like Ligand for DR3 and TR6/DcR3 and Functions as a T Cell Costimulator" which was published in the peer-reviewed scientific journal Immunity in March 2002 (Immunity, Vol. 16, 479-492). I will refer to this publication as "the Migone paper." A copy of the Migone paper is attached hereto as Exhibit B.

4. I am familiar with the publication entitled "DR3 Regulates Negative Selection during Thymocyte Development" authored by Eddie Wang et al., which was published in the peer-reviewed scientific journal Molecular and Cellular Biology in May 2001 (Mol. Cell. Biol., Vol. 21, 3451-3461). I will refer to this publication as "the Wang paper." A copy of the Wang paper is attached hereto as Exhibit C.

5. I am familiar with the publication entitled "Signal Transduction by DR3, a Death Domain-Containing Receptor Related to TNFR-1 and CD95" authored by Arul Chinnaiyan et al., which was published in the peer-reviewed scientific journal Science in

November 1996 (Science, Vol. 274, 990-992). I will refer to this publication as "the Chinnaiyan paper." A copy of the Chinnaiyan paper is attached hereto as Exhibit D.

6. I am familiar with the publication entitled "A New Death Receptor 3 Isoform: Expression in Human Lymphoid Cell Lines and Non-Hodgkin's Lymphomas" authored by Krzysztof Warzocha et al., which was published in the peer-reviewed scientific journal Biochemical and Biophysical Research Communications in 1998 (Biochem. Biophys. Res. Comm., Vol. 242, 376-379). I will refer to this publication as "the Warzocha paper." A copy of the Warzocha paper is attached hereto as Exhibit E.

7. I have been asked by patent counsel for HGS to provide my understanding of the correlation between: (a) the characterization of the DR3 molecule as indicated in the Application and the Migone, Wang, Chinnaiyan and Warzocha papers; and (b) the usefulness of the DR3 molecule as disclosed in the Application.

CELLULAR RESPONSES TO DR3 ACTIVATION

8. The Application discloses that DR3 is a TNFR family member containing a death domain and describes the role DR3 plays in mediating intracellular signaling which can lead to various cellular responses including, for example, apoptosis and cell proliferation. *See e.g.*, the Application at Page 5, line 15 through Page 6, line 27; at Page 29, lines 9-16; at Page 39, lines 3-11; and at Page 41, lines 11-13 and lines 21-25.

9. Prior to March 12, 1996 it was known that signaling through TNFR-1 and Fas, the two characterized TNFR family members known to have death domains, can cause both apoptosis and NF-κB activation, with the cell's commitment to either pathway

being determined by the intracellular balance between divergent signaling pathways. *See e.g.*, Hsu, H. et al. (1996). "TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways." Cell 84(2), 299-308, published January 26, 1996. A copy of the Hsu paper is attached hereto as Exhibit F.

10. Prior to March 12, 1996 it was known that activation of NF-κB is responsible for a variety of immune cell responses, including cell proliferation. *See e.g.*, Snapper, C. M. et al. (1996). "B Cells from p50/NF-κB Knockout Mice Have Selective Defects in Proliferation, Differentiation, Germ-Line CH Transcription, and Ig Class Switching." J. Immunol. 156, 183-191, published January 1, 1996. A copy of the Snapper paper is attached hereto as Exhibit G.

11. Accordingly, the assertion in the Application that signaling through DR3 may induce apoptosis and proliferation would have been credible to a scientist in the field of molecular biology in light of the state of knowledge in the art at the time of filing of the Application.

12. In Example 6 of the Application, overexpression of DR3 is shown to specifically induce apoptosis in MCF7 breast carcinoma cells. Overexpression of TNF receptor family polypeptides is an art accepted means of mimicking receptor activation in mammalian cell culture. *See e.g.*, Boldin, M. et al., (1995). "Self-association of the "death domains" of the p55 tumor necrosis factor (TNF) receptor and Fas/APO1 prompts signaling for TNF and Fas/APO1 effects." J. Biol. Chem. 270(1), 387-391, published January 6, 1995. A copy of the Boldin paper is attached hereto as Exhibit H. Therefore,

this data confirms the assertion of the Application, that DR3 activation can cause apoptosis in certain cellular environments.

13. In Example 6 of the Application, overexpression of DR3 is shown to specifically induce apoptosis and proliferation in 293 cells. *See*, the Application at Page 72, lines 23-25. Therefore, this data confirms the assertion of the Application, that DR3 activation can lead to an apoptotic response or a proliferative response depending upon the cellular environment.

14. The Migone paper demonstrates that: (a) recombinant DR3 specifically binds the TNF ligand TL1A and specifically induces activation of NF-κB when expressed in 293T cells; (b) endogenous DR3 specifically binds the TNF ligand TL1A and specifically induces activation of NF-κB in the erythroleukemic cell line TF-1 and also in activated T cells; (c) activation of endogenous DR3 by addition of TL1A in the presence of cycloheximide specifically induces caspase activity and apoptosis in the erythroleukemic cell line TF-1, but not in activated T cells; and (d) TL1A was more effective than FasL in inducing apoptosis of TF-1 cells under similar conditions.

15. The data and conclusions presented in the Migone paper are consistent with, and do not contradict, the function of DR3 disclosed in the Application. In its totality, the Migone paper supports the disclosure of the Application and confirms that the effects of DR3 activation are context specific and that DR3 can act to promote apoptosis in certain cellular environments.

16. The Wang paper describes the generation and characterization of mice that lack expression of the DR3 gene. The Wang paper identifies a non-redundant role for DR3

in the removal of self-reactive T cells in the thymus and in so doing identifies a physiological context in which DR3 is responsible for T cell apoptosis. The Wang paper does not exclude the likelihood of other redundant roles for DR3 in the regulation of T cell death.

17. The data and conclusions presented in the Wang paper are consistent with the function of DR3 disclosed in the Application. In its totality, the Wang paper confirms that DR3 does indeed mediate T cell apoptosis in at least one physiological circumstance.

18. The Chinnaiyan paper demonstrates that: (a) recombinant DR3 specifically binds the TRADD signaling molecule *in vitro* and when expressed in 293T cells; (b) recombinant DR3 can specifically activate NF-κB when expressed in 293 cells; (c) recombinant DR3 can specifically induce apoptosis when expressed in 293 cells; and (d) overexpression of recombinant DR3 specifically induces apoptosis in MCF7 breast carcinoma cells.

19. The data and conclusions presented in the Chinnaiyan paper are consistent with the function of DR3 disclosed in the Application. In its totality, the Chinnaiyan paper confirms that the effects of DR3 activation are context specific and that DR3 can act to promote apoptosis in certain cellular environments.

20. The experimental results presented in the Application, as well as those in the Migone, Wang and Chinnaiyan papers, support the functional role of DR3 as disclosed in the Application. These data, individually and in combination, confirm that DR3 can induce apoptosis under certain conditions and stimulate cell proliferation under other conditions.

TISSUE AND CELLULAR DISTRIBUTION OF DR3 EXPRESSION

21. From analysis of numerous experiments performed by myself or performed by other employees at HGS, it is my belief that:

(a) DR3 is expressed at very low levels in normal resting T cells; and

(b) DR3 is expressed at very low levels in normal B cells where it is sometimes undetectable.

22. The Warzocha paper shows that DR3 is "abundantly expressed" in a panel of pre-B acute lymphoblastic leukemia cell lines as well as in each of eleven distinct clinical isolates of follicular lymphoma. *See*, Page 377, lines 26-28 of the right column; and Figure 2. Therefore, Warzocha confirms that DR3 overexpression is useful as a diagnostic marker for certain lymphoid cancers such as acute lymphoblastic leukemia and follicular lymphoma.

23. The Application discloses that DR3 is expressed in lymphoid tissue and that increased expression of DR3 may be used to detect the presence of certain cancers including, for example, cancers of lymphoid tissue, such as follicular lymphoma. *See e.g.*, the Application at Page 5, lines 10-14; at Page 36, line 25 through Page 37, line 8; and at Page 38, lines 5-8; and at Page 38, lines 18-25.

24. In light of the observed expression profile of DR3, together with the experimental results presented in the Warzocha paper, one of ordinary skill in the art would find it credible that DR3 is useful as a diagnostic marker for certain cancers, as disclosed in the Application.

PHYSIOLOGICAL ROLES OF DR3

25. As declared above (*see*, statements 14 and 15), the Migone paper shows that DR3 can act to promote apoptosis under certain conditions. Furthermore, the Migone paper shows that: (a) DR3 activation of activated T cells *in vitro* via TL1A lead to increased IL-2 responsiveness and increased secretion of pro-inflammatory cytokines; (b) activation of DR3 promoted splenocyte alloactivation in an animal model of GVHD and in so doing increased the severity of the graft versus host response; and (c) a solubleTL1A-binding DR3 fusion protein can reduce TL1A stimulated apoptosis of TF1 cells in a dose-dependent manner.

26. The data and conclusions presented in the Migone paper confirm that: (a) regulation of DR3 activation would modulate the physiological response believed to underlie inflammation and inflammatory diseases such as GVHD; (b) regulation of DR3 activation would regulate the unfettered cellular proliferation that is believed to underlie the development of certain cancers; and (c) regulation of DR3 activation would modulate development of immune responses which are necessary to control viral infections and which underlie autoimmune diseases such as rheumatoid arthritis.

27. As declared above (*see*, statements 16 and 17), the Wang paper shows that DR3 does act to promote T cell apoptosis under certain physiological conditions. Furthermore, the Wang paper does not exclude the likelihood of other redundant roles for DR3 in the regulation of T cell death.

28. The data and conclusions presented in the Wang paper provide further confirmation that regulation of DR3 activation would modulate immune responses which

underlie inflammation and inflammatory diseases such as GVHD, control of viral infections and certain autoimmune diseases such as rheumatoid arthritis.

29. The Application asserts that DR3 polypeptides and antibodies are useful in the treatment of diseases such as cancers, including follicular lymphomas; inflammatory diseases, including Graft Versus Host Disease (GVHD); viral infections; and certain autoimmune disease such as rheumatoid arthritis. *See e.g.*, the Application at Page 6, lines 1-11; at Page 29, lines 9-17; at Page 38, lines 5-25; and at Page 46, line 20 through Page 47, line 3. It is my considered opinion that the Migone and Wang papers, together and individually, provide credible and compelling support for such uses of DR3 polypeptides and antibodies.

CONCLUSIONS

30. On reviewing the documents described above in their entireties, it is my considered opinion that: (a) the Application discloses functional characteristics of DR3 which were credible in light of the state of biological knowledge at the time the Application was filed; (b) the Application discloses functional characteristics of DR3 which have been corroborated by the work described in the Migone, Wang and Chinnaiyan papers; (c) the Application discloses that DR3 overexpression may be diagnostic for certain cancers, the credibility of this assertion is corroborated by the expression pattern of DR3 and the data reported in the Warzocha paper; and (d) the Application asserts that DR3 is useful in the treatment of certain cancers and inflammatory disorders, the credibility of this assertion is corroborated by the functional characterization of DR3 provided in the Application and the Migone and Wang papers.

31. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application captioned above or any patent issuing thereupon.

Date: 18 OCT 2002

Thi-Sau Migone Ph.D.